NIEHS News

Solutions from the Sea

Editor's note: This article on the Duke University Marine Biomedical Sciences Center (MBC) is the fourth in a series that appears intermittently in NIEHS News. The series highlights the activities of Environmental Health Sciences and Marine and Freshwater Biomedical Sciences Centers. The first article in the series appeared in volume 101, number 7.

The mission of the Duke University Marine Biomedical Sciences Center (MBC) is to use marine or freshwater organisms in research and training in the area of environmental health and to develop nonmammalian models and innovative methods for toxicological research. Its primary objective is to increase scientific and public understanding of the molecular mechanisms that underlie adverse environmental effects. The MBC acts as a bridge between the marine science community, the diverse programs of the School of the Environment at Duke University, and the clinical and research arms of the Duke University Medical School community.

Environmental pollution is a problem of increasing concern, one that is tightly linked to increases in world population. Human activities may be perturbing global weather patterns. The mental processes that make us creative and rational human beings can be impaired by toxic metals. We view with alarm the sores and cancers on fish taken from our lakes and oceans, sensing that their diseases forecast our own. There is a heavy reliance on pesticides, and DDT levels in our lakes and streams are rising. The programs at the Marine Biomedical Center (MBC) at Duke University play a role in solving such environmental problems.

Through its visiting scholar program, feasibility studies, thematic research, and outreach efforts, the MBC encourages research on mechanisms of toxicity. Understanding the complexities of these mechanisms requires knowledge of events that occur at the cellular or molecular level. Marine and freshwater organisms offer many simple and highly appropriate model systems that complement studies on vertebrates. Research using the sea urchin embryos, for example, have taught us about human growth and development. Millions of eggs are generated by a single female sea urchin during spawning, which occurs two or three times a year. The life and further development of the sea urchin is critically dependent on the integrity of the membrane that separates it from its external environment. Studies of sea urchin development have significantly advanced knowledge of how membranes are structured, how they allow access to some substances, exclude others, and the role they play in promoting or inhibiting growth and cellular differentiation. Scientists are also learning more about the way the cell membrane recognizes and responds to chemicals, knowledge that will enhance the ability to deliver medicines and target specific cells for treatment.

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Barnacles beware. Research on G-proteins in barnacles may give rise to nontoxic marine paints.

A Matter of Choice

Interactive multidisciplinary studies are the hallmark of MBC programs. Through interdisciplinary studies, it has been discovered that some of the metals previously regarded as inert are highly toxic, carcinogenic, or teratogenic in specific circumstances. Studies on the mechanism of toxicity of heavy metals, activated oxygen species, and free radicals are being pursued by MBC. Alternative animal models and new materials are being developed to better explore and prevent toxic environmental effects. The environmental effects of detergents is another area of study at MBC. In terms of molecular toxicity, detergents are not all alike. Although equal in cleaning efficiency, they vary widely in their effect on membrane structure and function. Studies of the relationship between detergent structure and environmental toxicity are in progress.

Interdisciplinary Research

Metal perturbation of development. David McClay studies the mechanisms of cellular differentiation and pattern formation, using sea urchin embryos as a model system. These researchers have recently discovered that nickel can cause developmental abnormalities. The effect of nickel is very specific. If embryos are treated with submillimolar concentrations of nickel during a specific time window, the embryos fail to establish normal dorsal and ventral axes. Most structures normally found in the "dorsal territory" fail to develop in the nickel-treated embryos. This finding has allowed McClay and colleagues to define several signals that operate during development and to explore the factors that influence cellular differentiation and commitment. McClay and colleagues are conducting an MBC-sponsored feasibility study to further clarify the effects of nickel.

Detergent toxicity. Celia Bonaventura and colleagues investigate the effects of various detergents on marine organisms. Results obtained to date show that oil consumption by a marine bacterium, *Achromobacter*, can be enhanced by adding detergent, probably as a result of increased bioavailability of the oil. At high levels, detergents can prevent bacterial growth by causing membrane disruption. The desirable levels of detergents to use in promoting oil consumption by bacteria vary widely depending on the nature of the detergent.

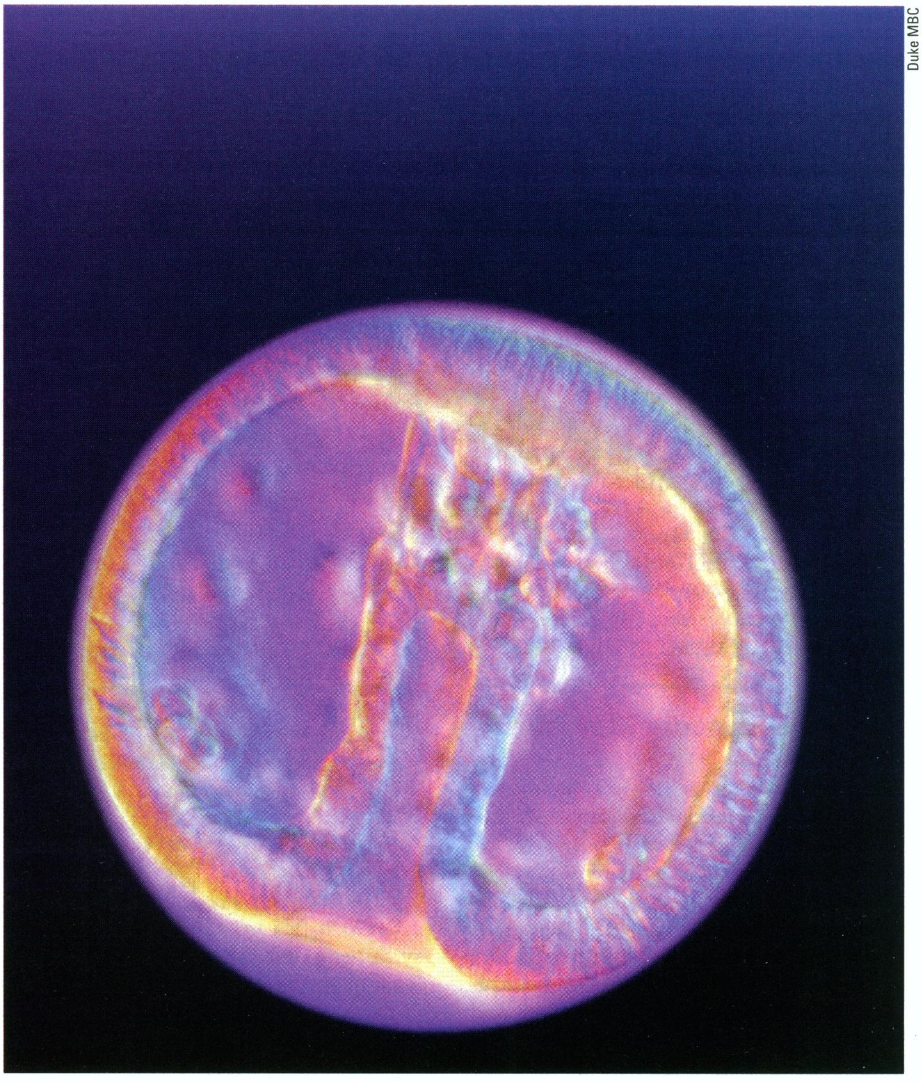
Although detergents are known to be acutely toxic to most aquatic organisms, little is known about their toxic effects at the cellular and molecular levels. Donovan Bodishbaugh, a doctoral candidate in the Integrated Toxicology Program at MBC, is studying mechanistic aspects of detergent toxicity. In fish, the primary target tissue of detergents is believed to be the gill. Toxicity is being investigated both *in vivo* and in an *in vitro* fish-gill model to better understand toxic mechanisms and structure–activity relationships of this diverse group of industrial chemicals. Results to date suggest that in fish and other aquatic animals impaired osmoregulation is an important toxic mechanism for detergents.

Cancers in clams and human health. Results of a feasibility study conducted by Rebecca Van Beneden have led to long-term NIH support for exploration of the links between cancers in clams and humans. Studies by Van Beneden and collaborators showed, for the first time, oncogene activation in a bivalve system. The increased incidence of cancers in clams and increased ovarian tumors in neighboring human populations suggests a common cause, possibly the exposure of both clams and humans to agrichemicals.

Cancer susceptibility in fish. Richard Di Giulio and collaborators are researching the mechanisms of aromatic hydrocarbon metabolism and cancer production, using the channel catfish and the brown bullhead catfish. These two fish share many physiological features and can live in the same environment, yet they show pronounced differences in cancer incidence when exposed to the same environmental pollutants. Microsomes of the bullhead catfish generate sixfold greater reactive oxygen species upon incubation with quinones than microsomes of the channel catfish and the brown bullhead catfish. It appears that the toxicity and mutagenicity of quinones and related environmental pollutants is due largely to their redox cycling and subsequent generation of toxic oxygen species.

Nitric oxide involvement in learning and memory. Using the octopus as an animal model, David Robertson and colleagues have made exciting discoveries that show nitric oxide has a significant role in learning and memory. They found that inhibition of nitric oxide synthase (NOS) prevents tactile learning in the octopus. Animals injected intramuscularly with an NOS inhibitor failed to learn a standard tactile discrimination task that control animals were able to learn. Additional experiments showed that the animals could learn again after the NOS inhibitor had dissipated. This report is the first of such findings in an invertebrate.

Engineered hemoglobins. Joseph Bonaventura leads a research project aimed at biomedical applications of structurally distinct hemoglobins, "sculpted" by site-directed mutagenesis to perform a variety of functions. One aspect of work with engineered hemoglobins concerns possible cancer therapy based on increased oxygen delivery to tumors. Mark Dewhirst, a specialist in hyperthermia and radiation oncology, along with Bonaventura and other MBC participants, is studying ways hemoglobins engineered to act like fish hemoglobins may facilitate oxygen delivery to tumors by changing pH and temperature sensitivity.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Walls of wonder. Membranes of sea urchin embryos may hold answers to human development.

Nontoxic antifouling coatings. MBC participants are seeking alternatives to toxic metals to protect submerged surfaces from the adverse effects of biological growth (fouling). Dan Rittschof receives research support from the Office of Naval Research and from paint companies, who share a keen interest in eliminating potent metallic and organic toxicants from marine paints. Most antifouling coatings contain slow-release toxicants, either organotins or copper compounds. Increasingly, these coatings are being banned. Rittschof and colleagues are finding natural products that can be economically used as alternatives.

Progress is also being made in defining the systems that trigger larval settlement, an essential part of the fouling process. A collaborative effort between Anthony Clare, Stephen Liggett, and Ronald Thomas involves potential G-protein-coupled receptors of barnacles. The G-protein-coupled receptors, which have important roles in humans, may be significant in triggering barnacle settlement. Results of these studies suggest that paints can be made that block the barnacle settlement system.

Metals and bioremediation. Metals and chelation effects may prove to have an important role in environmental management and environmental bioremediation. Richard Barber and other oceanographers are considering recent data that suggest oceanic phytoplankton growth can be increased by increasing iron availability. The complexation of iron with organic or inorganic materials is an important factor in iron bioavailability. From another perspective, studies in the laboratory of Alvin Crumbliss have drawn attention to the use of iron by microbes in the soil and in our bodies. Iron is acquired by microbial organisms by the excretion of iron-specific complexing agents known as siderophores. Crumbliss and colleagues have studied the kinetics and mechanisms of ligand-exchange reactions with iron complexes that are relevant to microbial iron transport. They have shown that metallic environmental pollutants such as aluminum can interfere with normal iron transport processes.

The MBC director, Celia Bonaventura, invites inquiries regarding the projects described above, potential animal models, or other aspects of the MBC. For more information or to receive the Center's newsletter, ENVIRONS, write to MBC, Duke University School of the Environment Marine Laboratory, 135 Duke Marine Lab Road, Beaufort, NC 28516-9721.

The Duke University Marine Biomedical Center

Overlooking North Carolina's Crystal Coast, the Marine and Freshwater Biomedical Sciences Center (MBC) is located on Pivers Island, adjacent to historic Beaufort. The MBC was founded in 1978 through a competitive grant provided by the NIEHS. Administrative offices and research facilities are housed within the Duke University School of the Environment Marine Laboratory.

MISSION

The mission of the MBC is to promote the use of marine and freshwater organisms in research and training in environmental health and to develop nonmammalian models and other innovative methods for toxicological research.

OBJECTIVES

The MBC's primary objective is to understand the mechanisms that underlie adverse environmental health effects. MBC programs promote research and communication in the areas of toxicology of metals and free radicals, developmental toxicology and carcinogenesis, and environmental toxicology and chemistry.

SUPPORT

Funding support is provided in the areas of technical assistance, feasibility grants, visiting scholars; meetings, seminars, and workshops; and equipment and facilities.

Cyanide Exposure May Affect the Brain

Moderate, chronic exposure to cyanide, a commonly used industrial compound, may cause cellular damage and lesions to the brain, characteristic of what might be seen in Parkinson's disease, animal studies supported by the NIEHS reveal. Cyanide is regularly used in the metal-processing industry. It is also present in cigarettes, as well as in the pits of apples, peaches, and apricots, but it is thought that the amounts of cyanide in these substances are generally too low to cause harm.

However, some industrial workers are typically exposed to cyanide on a regular, sustained basis, and some experts suspect this could alter brain function. Evidence from early animal studies suggest it may cause a loss of key brain chemicals.

Gary Isom and colleagues at Purdue University treated mice with moderate doses of cyanide twice a day for a week. Sixteen hours after the last dose was given, various measures of brain chemicals, blood, and behavior were taken and compared to the same measures in control mice. The researchers found that dopamine levels were significantly depleted in the treated mice due to a loss of brain cells. In some parts of the brain, dopamine levels declined by as much as 41%. Such changes did not occur in the controls.

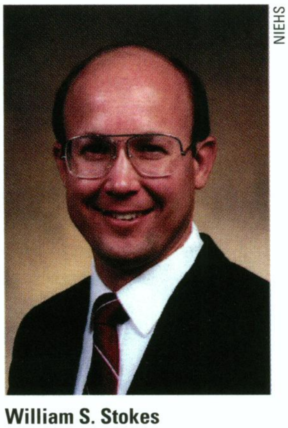
Dopamine is a neurotransmitter that helps regulate movement. Accordingly, mice treated with cyanide for longer periods of time experienced noticeable motor control deficits, such as incoordination. These effects were not seen in mice only briefly exposed to the agent.

The mobility impairment seen in the cyanide-treated mice mimics, in part, some symptoms seen in Parkinson's disease, leading the researchers to speculate that chronic exposure to elevated levels of cyanide may be a health hazard.

Validation of Alternative Methods

The NIEHS has established an ad hoc committee of staff from federal agencies that generate or use toxicity data for regulatory decisions. The committee will join the NIEHS in meeting the directive of the NIH Revitalization Act of 1993 to establish criteria for the validation and regulatory acceptance of alternative testing methods and to recommend a process through which scientifically validated alternative methods can be accepted for regulatory use. The panel has been designated the Interagency Coordinating Committee on the Validation of Alternative Methods, or ICCVAM.

In addition to the NIEHS, twelve other federal research and regulatory agencies have been invited to participate, including ATSDR, FDA, NIOSH and OSHA. The first meeting of the committee was in September, and it is anticipated that the group will meet monthly until completion of guidelines and recommendations on alternative methods.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William S. Stokes

William Stokes, a veterinarian on the NIEHS staff assigned to the alternative methods effort, noted that alternative methods, models, and approaches reduce the total number of animals required, incorporate refinements which result in the lessening of pain and distress to animals, and replace animals with nonanimal systems or replace one animal species with another, particularly if the substituted species is nonmammalian or invertebrate. "The vast majority of proposed new testing methods, both animal and nonanimal, usually involve some aspect of refinement, reduction, or replacement," Stokes said.

The committee's objective will be to develop uniform processes and criteria that will encourage the development of improved testing methods that will generate data more useful for risk assessment—lead to the scientific evaluation/validation of new alternative test methods, and increase the likelihood of regulatory acceptance of scientifically valid alternative test methods.